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Hepatitis B Vaccination in Patients With Chronic Hepatitis C

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The aim of the study was to evaluate the safety, immunogenicity, and possible therapeutic effect of hepatitis B vaccine in patients with chronic hepatitis C. The subjects studied included three groups: group I, 26 patients with chronic hepatitis C who were susceptible to hepatitis B virus infection; group II, 35 healthy subjects who were susceptible to both hepatitis B and hepatitis C virus infection; and group III, 30 patients with chronic hepatitis C receiving no hepatitis B vaccination as controls. Three 20 μg/dose of recombinant hepatitis B vaccines were given to subjects of groups I and II in months 0, 1, and 6. Blood samples from the subjects were collected before and 1 month after each dose of vaccination for serological testing. The subjects of groups I and II had similar antibody to hepatitis B surface antigen (anti-HBs) response rates after the first (30.8% vs. 17.1%), second (61.5% vs. 60.0%), and third (88.5% vs. 91.4%) doses of vaccination. Also, their geometric mean titers of anti-HBs did not differ much when vaccination completed in 7 months (360 vs. 581 mIU/ml). During vaccination period, patients with chronic hepatitis C demonstrated no significant change of serum cytokines and HCV RNA levels, but significantly lowered ALT levels after three doses of vaccination. Hepatitis B vaccination is safe and immunogenic in patients with chronic hepatitis C. It did not significantly affect their levels of HCV RNA, but tended to lower ALT levels. ***J. Med. Virol. 59:463–468, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: chronic hepatitis C; cytokines; HCV RNA; hepatitis B vaccine; immunogenicity

INTRODUCTION

Taiwan is an endemic area of hepatitis B virus (HBV) infection (its carrier rates were 15%–20% in general population) where chronic hepatitis B is the most important cause of liver cirrhosis and hepatoma [Sung, 1981]. Since the development of diagnostic kit for detecting hepatitis C virus (HCV) infection [Kuo et al., 1989], HCV was found to be another important etiology of chronic liver disease [Lee et al., 1991, 1992]. It has been estimated that about 1%–2% of the adult population in northern Taiwan have suffered from chronic HCV infections [Lee et al., 1991]. The prevalence is believed to be even higher in some townships of southern Taiwan (19%–37%) [Lu et al., 1997].

Although coinfection with HBV and HCV is rare [Liaw et al., 1982], superinfection with different hepatitis viruses is common in patients with chronic hepatitis B or C in Taiwan [Chan et al., 1991; Wu et al., 1994; Liaw 1995], which usually leads to more complications and even higher mortality [Chan et al., 1991; Fattovich et al., 1991; Fong et al., 1991; Feray et al., 1993; Wu et al., 1994; Liaw, 1995]. It is therefore imperative for patients with chronic hepatitis C to get protected against from HBV infection.

The immunogenicity and efficacy of hepatitis B vaccine in newborn babies, children, and adults have been well established [Lee et al., 1988; Lo et al., 1988; Chan et al., 1992], but the immune response to this vaccine is not satisfactory in patients with end-staged renal diseases and immuno-compromized hosts [Grob et al., 1983; Stevens et al., 1984; Jacobson et al., 1985]. It would therefore be interesting to do the same investigation in patients with chronic hepatitis C. While vaccines seemed to have therapeutic effects on chronic hepatitis [Cohen, 1994], the effect of hepatitis B vaccine on chronic hepatitis C, in light of serum HCV RNA titers, cytokines, and liver aminotransferase levels, deserves being explored more in depth.

MATERIALS AND METHODS

Study Subjects

The studied subjects included three groups: group I, patients with chronic hepatitis C who were susceptible

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to HBV infection; group II, healthy subjects who were susceptible to both HBV and HCV infections; and group III, patients with chronic hepatitis C but receiving no hepatitis B vaccination as controls. Subjects who were susceptible to HBV infection were those being sero-negative for hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), and antibody to HBsAg (anti-HBs). Subjects who were susceptible to HCV infection were those being seronegative for antibody to HCV (anti-HCV). Patients with chronic hepatitis C were those who were all serological positive for anti-HCV and/or HCV RNA and had serum alanine aminotransferase (ALT) levels of at least twice the upper normal values (> 90 IU/L) for at least two times within a period longer than 6 months and/or had liver biopsy showing evidence of chronic hepatitis. All the participating subjects were aged over 18 years. Six months prior to or during the study period, no participant received interferon, immunosuppressive agents, antiviral drugs, or other vaccination. The study was approved by the ethical committees of Veterans General Hospital-Taipei and the Department of Health, Republic of China. Consent forms were obtained from all participants.

Vaccination Protocol

Three doses of recombinant hepatitis B vaccine (Engerix-B, 20-μg HBsAg/ml/dose, SmithKline Beecham Biologicals, Belgium) were given intramuscularly in the deltoid region to subjects of group I and II in months 0, 1, and 6, respectively.

Follow-Up and Laboratory Tests

Blood samples from the subjects were collected before and 1 month after each dose of vaccination for serological testing. Before the study, the screening tests included serum HBsAg, anti-HBc, anti-HBs, anti-HCV, antibody to human immunodeficiency virus (anti-HIV), and ALT. For hepatitis B vaccinees of group I and II, serum anti-HBs titers and ALT levels were measured in months 1, 2, 6, and 7. Serum HCV RNA titers were also checked in months 0, 1, 2, 6, and 7 from subjects of group I. For the control subjects of group III, their serum ALT levels were measured in months 1, 2, 6, and 7; and HCV RNA titers were checked in months 0, 2, and 7. Serum cytokines [intercellular adhesion molecule-1 (ICAM-1) and tumor necrosis factor alpha (TNF-α)] levels were also measured in months 0, 1, 2, 6, and 7 from chronic hepatitis C patients of group I who received hepatitis B vaccination.

Serological HBV markers including HBsAg, anti-HBc, and anti-HBs (AUSRIA, CORAB, and AUSAB, Abbott Laboratories, Chicago, IL) were measured by radioimmunoassay. Serum anti-HCV (Abbott HCV EIA Second Generation, Abbott Laboratories) and anti-HIV (RAPID ELAVIA; Diagnostics Pasteur, Marnes-la-Coquette, France) were measured by enzyme-linked immunosorbent assay (ELISA) with commercial kits. Anti-HBs titers, expressed in mIU/ml, were calculated by the dilution method, with reference to a World Health Organization anti-HBs standard [Lo et al., 1988]. Titers of anti-HBs > 10 ml/mL were interpreted as protectively positive. Serum ALT was determined by a sequential multiple-channel analyzer with computer (Technicon, Tarrytown, NY). HCV RNA titers (Quantiplex HCV RNA 2.0 Assay; Chiron, Emeryville, CA) were checked by quantitative branched chain DNA (bDNA) assay [Chan et al., 1995]. Serum ICAM-1 and TNF-α were measured with Parameter ICAM-1 and Quantikine TNF-α ELISA kits (Research and Diagnostic Systems, Minneapolis, MN). The assays were based on the quantitative "sandwich" ELISA technique. A monoclonal antibody specific for ICAM-1 or TNF-α was coated onto the microtiter plate provided in the kits. Standards and samples were pipetted into the wells and any ICAM-1 or TNF-α present in the serum was bound by the immobilized antibody. After washing away any unbound proteins, an enzyme-linked polyclonal antibody specific for ICAM-1 or TNF-α was added to the wells to sandwich the ICAM-1 or TNF-α immobilized during the first incubation. Following a wash to remove any unbound antibody enzyme reagent, a substrate solution was added to the wells. Color then developed in proportion to the amount of ICAM-1 or TNF-α in the serum and was compared with the standard in optical density and concentration. Body temperature and possible side effects were recorded for 3 days after each dose of vaccination in subjects of groups I and II.

Statistical Analyses

Data in the text are expressed as mean ± SEM. Chi-square test or Mann-Whitney rank sum test was used to evaluate the significance of differences between groups. Wilcoxon signed rank test was used to evaluate changes of the serological parameters in the study period.

RESULTS

From September 1995 to June 1997, chronic hepatitis C patients who visited Gastroenterology Clinic, Veterans General Hospital-Taipei, were asked to participate in the study and received serological hepatitis B and C markers screening: group I consisted of 26 patients and group III consisted of 30 patients. In the same study period, 35 healthy subjects who were seronegative for HBV and HCV markers and received hepatitis B vaccination were listed in group II. Table I shows the demographic characteristics of the subjects enrolled in the three study groups. There was no significant difference in age and sex between groups I and III, but group II patients were significantly younger than group I and III ($P < 0.001$). All of the participants were seronegative for anti-HIV.

Immunogenicity of Vaccine

Subjects of group I and II showed no significant difference in anti-HBs response rates 1 month after the first (30.8% vs. 17.1%), second (61.5% vs. 60.0%), and third (88.5% vs. 91.4%) dose of vaccination. The geo-

TABLE I. Characteristics of Subjects at Enrollment

	Group I (n = 26)	Group II (n = 35)	Group III (n = 30)
Serological hepatitis markers	Anti-HCV(+), anti-HBc(−)	Anti-HCV(−), anti-HBc(−)	Anti-HCV(+), anti-HBc(+)
Mean age (range), year	47.3 (19–68)[a]	33.7 (18–68)[a]	48.9 (20–70)[a]
Sex (male/female)	9/17	18/17	10/20
Hepatitis B vaccination	Yes	Yes	No

[a]$P < 0.001$ by Mann-Whitney ranked sum test.

metric mean titers of anti-HBs were also not significantly different in both groups after completion of vaccination in month 7 (360 vs. 581 mIU/ml; Table II); 65.4% (17/26) of group I chronic hepatitis C patients at this time had an anti-HBs titer above 100 mIU/mL, which was similar to 71.4% (25/35) of group II healthy subjects obtained anti-HBs titer greater than 100 mIU/mL.

Reactogenicity of Vaccine

No serious adverse effects were found during the vaccination period. As shown in Table III, the majority of symptoms, local or general, were mild in intensity and all subsided within 1 day. There was no statistically significant difference in incidence of side effects between two groups of vaccinees, but chronic hepatitis C patients are more likely to experience malaise than healthy subjects.

Changes of Liver Enzymes, Cytokines, and Viral Markers

Fifteen patients in group I and 20 patients in group III had detectable HCV RNA in their sera in month 0. Serum HCV RNA was not lost in any patients, with or without hepatitis B vaccination. During the study period, as shown in Figure 1, serum HCV RNA levels were not changed significantly in chronic hepatitis C patients despite having hepatitis B vaccination or not, but mean ALT levels were found to lower significantly in patients who received hepatitis B vaccination (group I) in month 7 as compared with the month 0 (62.9 ± 10.9 vs. 101.9 ± 21.5 IU/L; $P = 0.01$). Before the vaccination, the mean levels of serum ICAM-1 (775.8 ± 72.5 vs. 415.0 ± 22.1 ng/ml, $P < 0.001$) and TNF-α (7.56 ± 0.45 vs. 6.77 ± 0.21 pg/ml; $P = 0.082$) were found to be higher in chronic hepatitis C patients (group I) than in healthy subjects (group II). During the follow-up period, however, serum ICAM-1 or TNF-α levels were also not significantly changed in chronic hepatitis C patients who received hepatitis B vaccination (group I; Fig. 2).

DISCUSSION

It has been reported that the immune response to hepatitis A vaccine in patients with chronic hepatitis B or C is slightly inferior to that in healthy subjects [Lee et al., 1996, 1997; Keeffe EB et al., 1998]. The immune response to hepatitis B vaccine was also found to be lower in hemodialysis patients who were positive for anti-HCV [Navarro et al., 1996], probably because hemodialysis patients had low immune response to hepatitis vaccine [Stevens et al., 1984]. The controlled study of Cheng et al. [1997] found the same immune response to hepatitis B vaccine in hemodialysis patients who were either anti-HCV positive or negative. Kamel et al. [1994] also reported that HCV infection would not affect the immunogenicity of hepatitis B vaccine in healthy subjects. Our present controlled study disclosed similar anti-HBs response rates between chronic hepatitis C patients (group I) and healthy subjects (group II) on standard schedule of hepatitis B vaccination. The anti-HBs titers were also not significantly different between these two groups. Having completed vaccination, 88.5% of chronic hepatitis C patients and 91.4% of healthy subjects had anti-HBs seroconversion (anti-HBs titer > 10 mIU/mL). Effective immunity (anti-HBs > 100 mIU/mL) was observed in 65.4% of chronic hepatitis C patients and in 71.4% of healthy subjects. In fact, age was a factor of the immunogencity of hepatitis B vaccine: older subjects had lower immune response than younger ones [Hollinger, 1989]. Nevertheless, our finding of much older chronic hepatitis C patients compared with healthy subjects demonstrated that chronic hepatitis C patients did not necessarily have poorer immune response to hepatitis B vaccination than healthy subjects.

The pathogenesis of HBV and HCV is still poorly understood. The coexistence of both viruses in subjects may arouse complicated interaction [Liaw, 1995]. For example, changes of virological profiles and/or disappearance of specific viral antigens were found clinically in chronic viral hepatitis patients with multiple hepatitis virus infections [Bradley et al., 1983; Dienes et al., 1990; Liaw et al., 1991; Sheen et al., 1992; Pontisso et al., 1993]. It has been reported that hepatitis B vaccination may also have therapeutic effects on patients with chronic hepatitis B [Cohen, 1994]. Thus, our observation of changes of serum ALT, cytokines, and HCV RNA levels in chronic hepatitis C patients with hepatitis B vaccination may give us some hints of its possible therapeutic effects.

The role of cytokines in the pathogenesis of viral hepatitis has been studied by many investigators, e.g., TNF-α has multiple inflammatory and immunoregulatory properties, increased production by mononuclear cells of the liver, and peripheral blood was found in patients with chronic hepatitis C [Yoshioka et al., 1989, 1990; Lu et al., 1995]. ICAM-1 is an important early marker of immune activation and response. Measurement of a soluble form of ICAM-1 may help investigate

TABLE II. Immunogenicity of Hepatitis B Vaccine in Susceptible Chronic Hepatitis C Patients and Healthy Subjects[a]

Vaccinees	Total cases	Anti-HBs response, months			
		1	2	6	7
Group I, chronic hepatitis C	26				
Number (%) positive		8 (30.8)	16 (61.5)	15 (57.7)	23 (88.5)
GMT (mIU/ml) of responders		63	40	72	360
Group II, healthy subjects	35				
Number (%) positive		6 (17.1)	21 (60.0)	23 (65.7)	32 (91.4)
GMT (mIU/ml) of responders		47	45	76	581

[a]Anti-HBs, antibody to hepatitis B surface antigen; GMT, geometric mean titers of anti-HBs.

TABLE III. Side Effects of Hepatitis B Vaccination in Patients With Chronic Hepatitis C and Healthy Subjects[a]

Vaccinees	Total cases	Total vaccine doses	Number (%) with side effects		
			Local		General malaise
			Pain	Swelling	
Group I, chronic hepatitis C	26	78	8 (10.3)	3 (3.8)	11 (14.1)
Group II, healthy subjects	35	105	9 (8.6)	2 (1.9)	7 (6.7)

[a]No statistical significant difference between two groups. No redness, fever, headache, or gastrointestinal symptoms.

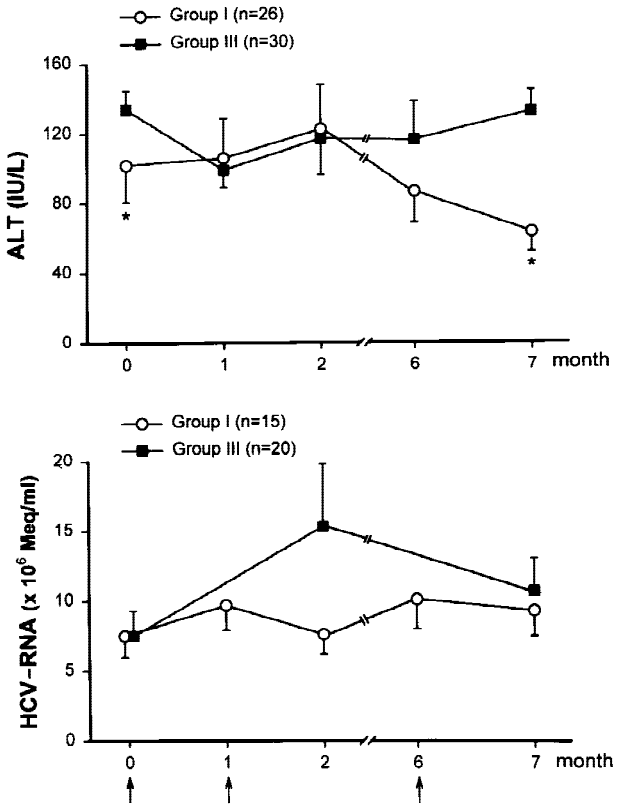

Fig. 1. Changes of serum ALT (alanine aminotransferase) and hepatitis C virus (HCV) RNA levels in chronic hepatitis C patients receiving hepatitis B vaccination (group I) or not (group III). Data expressed as mean ± SEM. Arrows mean the time when hepatitis B vaccine was given.

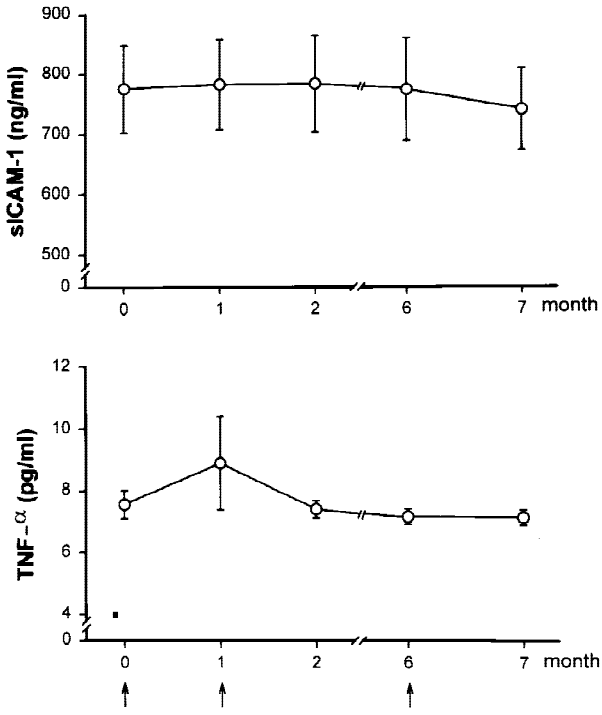

Fig. 2. Changes of serum cytokines (ICAM-1 and TNF-α) levels in chronic hepatitis C patients receiving hepatitis B vaccination (group I, n = 26). SICAM-1 = serum intercellular adhesion molecule-1; TNF-α = tumor necrosis factor alpha. Data expressed as mean ± SEM. Arrows mean the time when hepatitis B vaccine was given.

and monitor various inflammatory, neoplastic, and immune disorders [Ninova et al., 1995; Pirisi et al., 1997; Taketomi et al., 1997]. Patients with chronic liver disease have shown elevated serum levels of ICAM-1 that have a direct relationship to the severity of the disease [Pirisi et al., 1997; Taketomi et al., 1997]. Our present study also demonstrates increase of both serum TNF-α and ICAM-1 levels in chronic hepatitis C patients as compared with healthy subjects. Although the mechanism of liver damage in chronic hepatitis C is still unknown, several humoral factors, including some cytokines, may be involved. If hepatitis B vaccination can influence the production of cytokines, e.g., TNF-α or ICAM-1 in peripheral blood mononuclear cells, it may provide a therapeutic effect on the hepatocytes through

the interaction of these cytokines, even though it does not change serum HCV RNA levels. However, our data did not support this hypothesis: neither serum HCV RNA nor cytokines was changed in chronic hepatitis C patients receiving hepatitis B vaccination.

One of the primary objectives of the present study was to evaluate the safety of recombinant hepatitis B vaccine in patients with chronic hepatitis C. As shown in Table III, the incidence of reactogenicity, either local or general, was not significantly different between chronic hepatitis C patients and healthy subjects receiving hepatitis B vaccination, although chronic hepatitis C patients had more complaints of malaise probably due to the disease itself. The vaccine also did not exacerbate the liver aminotransferase levels; significantly lower ALT levels were found in chronic hepatitis C patients who received hepatitis B vaccination.

In conclusion, hepatitis B vaccine is safe and immunogenic in patients with chronic hepatitis C. It does not significantly affect the patients' serum HCV RNA and cytokines levels, but tends to lower ALT levels significantly in 7 months—1 month after booster dose of vaccination.

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